3D-Printed Osteo-Electro Hydroxyapatite-Graphene Composites

Adam E. Jakus, Ramille N. Shah
Simpson Querrey Institute for BioNanotechnology, Materials Science and Engineering, Northwestern University

Statement of Purpose: There is no longer any significant doubt that 3D-printing will play a significant role in the future of medicine. We introduce a particle-based 3D-ink preparation and 3D-printing method, based on Hyperelastic Bone (presented at SFB 2014), that is extended to create hydroxyapatite (HA)-graphene (HAG) inks that can be rapidly 3D-printed into highly electrically conductive and bioactive scaffolds. The osteogenic bioactivity of HA is well documented, and the electrogenic capacity of graphene is becoming a heavily researched area of tissue engineering and biomaterials, with evidence indicating that graphene can enhance differentiation towards and function of electrogenic cell types such as myoblasts, neurons, and cardiomyocytes. Musculoskeletal tissues require simultaneous and integrated osteogenic and electrogenic functions and potential. We show in this work that 3D-printed HAG's novel osteo-electro properties make it a promising musculoskeletal biomaterial system.

Methods: All particle-based inks discussed in this work were synthesized via suspension, dissolution, and agitation of 60-70 vol.% (solids: polymer + powder) powders in solutions containing 30-40 vol.% biocompatible elastomer dissolved in a graded solvent mixture. Where "powders" refers to hydroxyapatite (HA), graphene, and combinations of HA and graphene. All inks were 3D-printed into constructs for electrical, mechanical, and *in vitro* testing using a 3D-Bioplotter (EnvisionTEC, GmbH), an extrusion-based platform. The microstructure of 3D-printed constructs were investigated using scanning electron microscopy (SEM). Quasi-static compressive and tensile mechanical testing were performed on 3D-printed cylindrical and "dog-bone" specimens, respectively. Electrical resistivity measurements were performed using a 4-point probe setup. *In vitro* experiments were performed by seeding human mesenchymal stem cells (hMSCs) onto washed and sterilized 3D-printed, porous scaffolds and cultured for up to 2 weeks. Confocal fluorescent and SEM imaging were used to observe cell viability and morphology. DNA quantization and gene expression analysis through qPCR at multiple time points were also performed.

Results: Despite containing as much as 70 vol.% solid particles, composites comprised of 1:1 (by volume) HA-graphene can be 3D-printed into substantial objects, such as the 100+ layered porous cylinder shown in Figure **1A**. Although of a macroscopically significant scale, HAG objects retain high fidelity (**1B**) and user-defined pore structure throughout the entire object volume. SEM imaging (**1C**) reveals that graphene dominates the surface of HAG and encapsulates the larger HA particles. hMSCs both adhere to and proliferation on HAG scaffolds (**1D**). (**1E**) After 14 days within the HAG scaffolds, hMSCs coat most surfaces and begin filling the void between HAG struts. Closer observations indicate that hMSCs are closely interacting with graphene surface and tend to have an affinity for regions with underlying HA particles (**1F**). Based on cell morphology and preliminary gene expression analysis, there is reason to believe that both osteogenic and electrogenic relevant genes of the hMSCs are being upregulated over the course of the two weeks in culture.

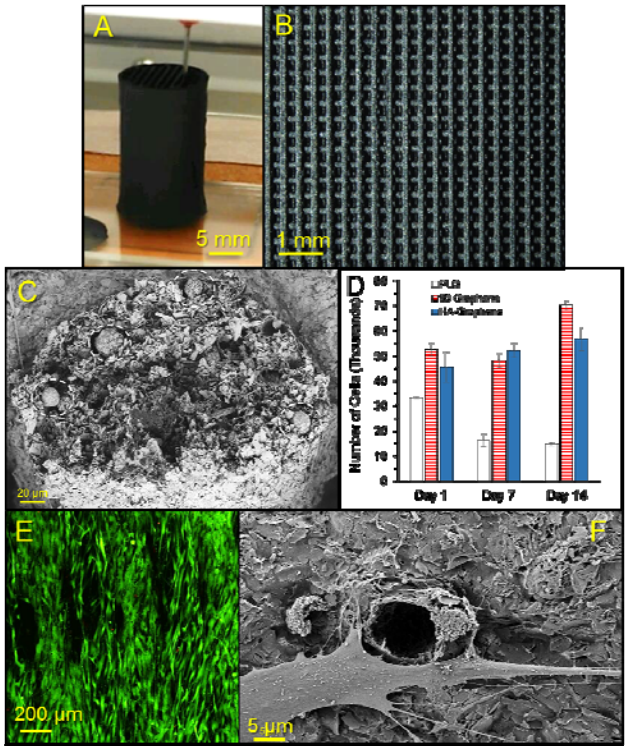

Figure 1. (**A**) photograph of HAG being 3D-printed into a 100+ layer cylinder using a 250 µm diameter tip. (**B**) optical photograph of HAG, top-down-view, illustrating that a high degree of user defined periodicity can be retained. (**C**) SEM micrograph of cross-section of single HAG strut within larger 3D-printed construct. White circles show HA particles. (**D**) hMSC number as a function of time after seeding onto 3D-printed PLG, 20% Graphene, and HAG scaffolds, as determined through DNA quantification. (**E**) Laser-scanning Z-stack projection of 3D-reconstruction of Live (green) Dead (red) stained HAG scaffold 14 days after being seeded with hMSCs. (**F**) SEM micrograph of single hMSC on graphene dominated HAG surface near a void which was previously occupied by an HA particle.

Conclusions: These initial studies confirm that HA-graphene composite structures can be rapidly fabricated using extrusion-based 3D-printing under ambient conditions. Additionally, preliminary evidence suggests that seeded stem cells are positively responding to the osteo-electro nature of the material, making a HAG a promising new musculoskeletal biomaterial worthy of future functional studies.